Original study protocol

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Project Title: Correcting the Deficiency of Dietary Dairy Produce in the Elderly Reduces Fractures and Preserves Bone Strength

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Background

The problem - fractures in the elderly

Fractures in the elderly result in increased morbidity, mortality and health costs. By 2051, the number of Australians aged over 65 years will double and will increase the absolute number of fractures and the burden of these fractures to the community. The annual cost of fractures in Australia is estimated at $7.4 billion (1-3). Given the growth of the aging population these costs will escalate. About 65% of all fractures in the community occur in persons over 65 years of age. The incidence of fractures is 9-14% in aged care facilities that currently accommodate in excess of 150 000 elderly Australians (4-7). Of all the hip fractures in the community, 30% come from elderly in aged care, especially from ambulatory low level care residents who have the highest fracture rates in the community (8, 9)**.** Targeting those in aged care is likely to assist in reducing the burden of fractures in the community and is likely to be cost effective as this identifies a group at high risk of fracture likely to benefit from intervention.

The cause of the problem – poor nutrition in the elderly

Nutritional deficiencies contribute to fracture risk. The burden of fractures attributable to calcium and protein under nutrition is large because many people have low intakes. 43% of elderly living in the community receiving home assistance and 65% of low-level care residents are malnourished or at risk of malnutrition (10, 11). We report that in ambulatory aged care residents 100% of women and 84% of men consume less than the recommended amount of 1300mg/day with 68% of women and 16% of men consumed less than 600 mg/day of calcium (11). These rates of dietary calcium deficiency are similar to elderly in the community (12, 13). In aged care, 77% of residents consume below the suggested requirement of > 1g protein/kg body weight (14, 15). In many cases the actual foods served in aged care facilities do not provide sufficient nutrients (11).

Calcium deficiency contributes to fracture risk. Women with reported lactose intolerance, who avoid consuming milk, have lower dairy calcium intakes than women who drink milk (570mg/day v 850 mg/day) and a 33% greater risk of fractures (16). Dietary calcium deficiency has been shown to increase bone remodelling intensity and as each remodelling unit removes more bone than it deposits, each of the many remodelling events erodes bone resulting in increased cortical porosity, cortical thinning, and loss of trabecular connectivity. This structural decay predisposes bone to fractures (17).

Protein deficiency also contributes to fracture risk. Low protein intake is associated with bone loss, reduced bone mass and strength and increased fracture risk (18-21). Malnutrition is associated with reduced bone formation by each basic multicellular unit (BMU) so when bone resorption occurs less bone is deposited by the BMU. Reduced IGF-1 and other factors contribute to the reduction in bone formation (20, 22, 23). Protein deficiency reduces IGF-1 levels and low IGF-1 levels are associated with increased fracture risk in post-menopausal women (24, 25).

The solution - correcting calcium and protein deficiency

The burden of fractures is too large for drug therapy – trials in the elderly are limited, treatment is not validated and difficult to administer and has serious side effects. Treatment given as prevention will cost more than the disease.

The solution to the burden is a public health approach that is effective, safe, easily administered, readily available to all persons and low cost. Of the two options available – increasing dairy food intake and exercise – only the former fits the requirement. All the criteria apply to dairy foods but studies of dairy foods and fracture risk reduction have not been undertaken.

Studies of calcium supplementation with or without vitamin D have been done but they are seriously flawed in their design; in virtually all of these studies, the participants were not calcium deficient and so it is not possible to either demonstrate a deleterious effect of a deficiency state nor the beneficial effect of repletion (17). The studies are also flawed in execution with poor compliance in about 50% of participants and 50% dropout rates so inferences cannot be made (17). In post hoc subgroup analyses, a beneficial effect of calcium is observed in high risk individuals such as those with low calcium intakes and those with prior fractures (seen in 30% of our sample of aged care residents) (26-28). These observations are hypothesis generating, not hypothesis testing. Calcium supplementation reduces secondary hyperparathyroidism, remodelling rate and so, bone loss (29**).** For protein, correcting protein deficiencies increases IGF-1, improve muscle mass, reduces falls, increase bone formation and may reduce the negative BMU balance and so bone loss (22). Protein supplementation trials have not been done in elderly persons however, inadequate protein intakes have been reported in hip fracture sufferers and protein supplementation improved serum IGF-1 levels and reduced recovery time and the incidence of complications during hospitalisation (30-32).

While no studies of fracture risk reduction using dairy produce have been done, there is other work that supports the need for this study (33-36). Bonjour et al observed a 12.3% decrease in PTH and a 16.9% increase in IGF-1 in elderly women after 1 month of supplementation with vitamin D / calcium fortified cheese (33). Manios et al supplemented post-menopausal women for five months with either calcium/vitamin D fortified dairy foods, calcium alone or they received no intervention, and observed improved IGF-1 levels in the intervention group relative to the calcium only or control groups ($p < 0.05$) (34).

The logic of studying fractures in a high-risk hostel setting

Fracture is the correct endpoint to demonstrate efficacy but as fractures are less common in the community (that nevertheless contribute to the burden because of the large numbers alone) the test of efficacy requires a population with a high baseline incidence of fractures such as low-level aged care residents (9). This information can be extrapolated to the community provided that the structural abnormalities leading to fractures and response to treatment are similar in community dwellers and aged care residents. We acknowledge it is an extrapolation. However, this limitation applies to all clinical research because clinical trials are done in a sample from which inferences are made to the community. Drug trials have strict inclusion criteria because what is being tested is efficacy of a treatment and that must be done under controlled conditions where influential covariates are equally present in treated and control groups. If efficacy is demonstrated the assumption is that this will be the case in the whole community in whom the drug will be used.

The results of the study by Chapuy et al have withstood the test of time (6). This study involved elderly women in nursing homes and is the only one that has convincingly shown the use of calcium and vitamin D to prevent fractures. The study we propose is similar but we will use dairy foods. We believe we can also achieve lasting and convincing results provided the design and execution

are given meticulous attention. The sample of elderly residents in aged care will not differ substantially to similarly aged elderly living in the community with similar numbers of chronic health conditions reported, and the study environment including food intake is stringently monitored and controlled (37).

We propose that improvements in porosity, trabecular density, remodelling markers and PTH will occur. Favourable changes in biochemical indices of bone metabolism have been observed with dairy-based interventions of durations as low as 1 month (33, 34). The bone structural changes and specifically the measurement of cortical porosity proposed in this study can only be investigated at our centre as we are the only centre in Australia with a high resolution peripheral quantitative computed tomography HRpQCT, and the patented STRAX 1.0 software that measures cortical porosity as developed by our team. We have recently published on the role of cortical porosity on bone strength and fracture risk, which has received an editorial in The Lancet (38). The inferences from these changes to cortical porosity, bone structure and biochemical markers of bone metabolism will be that these changes produce a fracture risk *reduction* in the community but this cannot be confirmed by counting fracture rates in the community as fracture endpoints will require thousands of people.

Dairy food consumption and cardio-vascular disease

Cardiovascular disease (CVD) (heart, stroke and blood vessel disease) remains the principal cause of mortality in Australia, responsible for 34% of all deaths in 2008. The majority of these deaths (75%) are in those over the age of 75 years. Ischaemic heart disease is one of the two principal causes of disability in the Australian population. High blood pressure is a major risk factor for heart disease and stroke.

The effect of dairy food consumption on CVD has not been demonstrated in randomised controlled trials. Evidence from prospective cohort and observational studies indicate that increased dairy food consumption is associated with reduced risk of mortality and stroke (39). This is further supported by a recent review of prospective cohort studies that did not observe increased coronary heart disease with dairy food consumption (40). Some of the CVD risk reduction may result from the blood pressure lowering effects observed with dairy food intake. Inverse relationships have been observed between dairy food intake and blood pressure, which may relate to dairy peptides, or the effects of the specific nutrients, calcium, potassium and magnesium found in dairy foods, which have been shown to moderately reduce blood pressure (41, 42).

In contrast, a recent meta-analysis indicated that calcium supplements may increase myocardial infarction (MI) rates by 30% however, the level of significance was marginal with the absolute rates being 2.7% versus 2.2% (43). The data for both stroke and all-cause mortality was not significant. Thus the only suggestion of significance is for non-fatal myocardial infarction, which is now defined using only a small rise in blood troponin level that is not necessarily associated with subsequent disability. The evidence available from observational studies of dairy food consumption does not support the observation of increased MI rates suggested with calcium supplementation, but this has not been tested in a randomised controlled trial.

Dairy food consumption, sarcopenia and mortality

Body composition changes with ageing, even in relatively healthy elderly. These body weight changes reflect changes in body composition as fat mass (FM) gradually increases while lean tissue mass (LTM) decreases. The marked age-related loss of skeletal muscle mass (SMM), termed sarcopenia, is associated with loss of strength, functional impairment, disability and increased mortality. Body composition assessed by densitometry is the preferred method of assessing

sarcopenia. Protein deficiency is associated with reduced IGF-1 and lean muscle mass (20, 44). Supplementation of older women with 15 grams of essential amino acids increased IGF-1 expression, lean muscle mass and muscle protein synthesis, and muscle protein synthesis rates are enhanced by leucine found in whey protein (45, 46). There is limited data investigating the role of dairy food in the prevention of sarcopenia. However, recent recommendations suggest that the progression of sarcopenia can be slowed and muscle protein synthesis maximised if larger quantities (e.g. 25-30g) of high quality proteins are consumed per meal (47). Dairy foods are a good source of protein and specifically, are a source of leucine, therefore these recommendations can be achieved by the inclusion of dairy foods with meals as part of an overall strategy to increase dairy food intake to recommended levels to help slow the onset of sarcopenia and reduce the incidence of malnutrition in aged care residents. Sacropenia was observed in 21% of women and 43% of men living in low level aged care facilities and 60% had two or more indices of malnutrition (11).

To date there has not been a randomised controlled trial in elderly people of a sufficient size or duration or with adequate power to clearly demonstrate that dairy food consumption does not increase the risk of CVD and mortality in elderly people. The proposed trial would provide this evidence, as this current study provides a unique opportunity to examine enhanced dairy food consumption in a prospective randomised trial, using non-inferiority (or equivalence) statistical methods, with the hypothesis that enhanced dairy food consumption in the elderly have no significant detrimental effect on cardiovascular events and will not increase mortality rates, while potentially reducing fracture rates.

Significance

An effective means of reducing fracture risk is to shift the population mean for dairy food intake in the elderly so a smaller proportion are deficient in calcium and protein. Blood pressure has been lowered by reducing salt intake in the whole population and is seen as an effective means of reducing the burden of stroke and cardiac events (48, 49). The principle of using dietary modifications to reduce cardiac events instead of using drugs can be applied to increasing dairy food intake to reduce fractures, and is a realistic option for fracture prevention in the community.

To justify making public health policy concerning the widespread increase in dairy food intake in the community, anti-fracture efficacy must first be demonstrated in a controlled trial. This will be achieved by testing the benefits of enhanced dairy food intake in ambulatory aged care residents – a group at high risk of fractures and with calcium and protein deficiencies; this has never been done in any study. Indeed, targeting of a high risk population has never been done – in all cases (except in the Chapuy et al study), patients have generally been calcium replete and the negative results are likely to be due to this fatal flaw in design – benefit of a treatment in correcting the effects of a deficiency state cannot be demonstrated if there is no deficiency to begin with (6).

While fracture risk in an individual attributable to calcium or protein deficiency alone is small, the high prevalence of deficiency confers a high attributable risk so shifting the population to a higher level of dairy food intake will have a large net effect (12, 13). For example for a 2% incidence rate of fractures (I = 0.02) and the estimation that 50% of elderly Australians are calcium deficient (p = 0.50), the relative risk of fractures associated with low calcium intake is 1.67 (RR = 1.67) or low calcium consumers have a 67% greater risk of fractures. From these numbers, we can estimate that the number of fractures in the low calcium group is I1 = 0.025 and in the high calcium group is I0 = 0.015. Assuming the efficacy (in relative risk reduction unit) of enhanced dairy intake is 20% (E = 0.2) the proportion of fractures we can prevent is: 0.015*1.67*0.5*0.2 / 0.02 = 0.125 or 12.5%. With nearly 3 million people over the age of 65

years currently in Australia, this equates to a reduction in the yearly medical cost of fractures alone of more than $40 million (3).

Evidence of the anti-fracture benefits from consuming dairy foods obtained from a well controlled and executed trial, will support the advocacy for a minimal dairy intake requirement in all aged care settings and other food providers for the aged, and strengthen the promotion of dairy food intake in the elderly. The advocacy for increased dairy food consumption in the elderly can further be enhanced if a decrease in risk of malnutrition, CVD and mortality is also observed.

Hypothesis and project objectives

Supplementation of aged care residents deficient in dairy intake (≥ 2 serves per day) will correct protein and calcium intakes to recommended levels (> 1g/kg weight for protein, > 1300 mg calcium / day).

Primary outcomes

i) Reduce fractures.

Secondary outcomes

ii) Slow bone remodelling.

iii) Reduce cortical porosity and thinning and maintain trabecular architecture.

iv) Reduce falls.

v) Reduce PTH, and increase serum IGF-1.

Tertiary outcomes

vi) Reduce cardio-vascular and all-cause mortality

vii) Reduce cardio-vascular events

viii) Reduce blood pressure and risk of hypertension

ix) Slow sarcopenia

x) Reduce malnutrition risk

Project design and methods

This 2-year intervention provides 4 serves of dairy foods per day in low-level aged-care residents. Residents are generally ambulatory and able to self-feed therefore differ from those in nursing homes whom are predominantly bed ridden and require assistance with most task. Facilities are matched by location and are allocated to intervention (enhanced dairy intake) or control (existing menu and current dairy intake) using randomly generated numbers. The demographics of residents in facilities in close proximity to each other, in relation to age, health status, BMI, sex, etc are similar so will be equally represented in each study arm. Residents are notified during their residents meetings that the facility is involved in the project and they have a 50% chance of being provided with additional dairy foods. Once allocated, only the principal investigator (SIB) and catering consultant are aware of group allocations - staff involved with testing are blinded to grouping. Management but not residents will be privy to the changes.

The catering consultant will work with food service staff to assist with menu modifications to incorporate sufficient serves of dairy into the daily menu. Changes include:

- Substituting afternoon tea of cake or sweet biscuits with cheese and crackers
- Using milk in place of water for hot chocolates
- Breakfast cereal / porridge using milk
- Using cheese sauces with meals
- Having custard as part of dessert

- Including cheese in sandwiches

Once the changes are in place it is not anticipated that staff changes will raise issues with food delivery as the menus are standardised and no specific preparation is needed to administer the foods.

Energy intake will not vary substantially because of the intervention, as high energy, nutrient poor foods such as sweet biscuits will be substituted with high energy, nutrient rich dairy foods. Intakes of other nutrients contained in dairy foods such as zinc and magnesium may also improve; these changes will be monitored using 3 day weighed food intakes. Moreover, we reported that energy intake was below requirements in females, zinc low in males and magnesium low in all aged care residents so it is likely that intakes of these nutrients will also improve (11). The additional dairy foods will be purchased by each facility via their usual provider. The additional dairy foods will be itemised on a separate invoice and forwarded to the project manager on a monthly basis for reimbursement.

Inclusion criteria:

(i) Intake < 600 mg calcium per day, < 1g / kg BW of protein
(ii) Not hypogonadal (testosterone levels > 10 nM in males)
(iii) Not vitamin D deficient (serum 25(OH)D levels > 25 nmol/L)
(iv) Free of disease or medication (e.g. bisphosphonates) use known to affect bone

If participants are identified as vitamin D deficient (< 25 nmol/L) the director of nursing will be notified and treatment initiated.

A menu assessment will be performed to determine dairy food availability. Dairy intake will be assessments using 3-day weighed food intakes and will be completed in all residents in all facilities prior to commencement of the trial (inclusion criteria < 600 mg/day or ~ 2 serves of dairy). Consent will be sought by residents to measure food intake. All residents will be supplemented (via the food service). Analysis of data will be restricted to those who meet the inclusion criteria.

We have conducted a feasibility study (Project No. H2010/04129) that demonstrated that the elderly aged-care residents were able to consume the two additional serves of dairy food per day (Iuliano et al, JNHA, accepted Nov 2012)

Nutrient intake and compliance The provision of dairy foods will be determined by inventory of dairy foods to each facility divided by number of residents. The nutritional quality of foods provided will be determined by a nutritional analysis of the menu at each aged care facility. Menus are rotated though regular monthly menu cycles. Compliance will be determined on a minimum of 10 residents per facility (e.g. 600 residents) using the method of duplicate weighed records, including records of uneaten or left over foods which is ~ 5% (11). This will be done at baseline and on 3 occasions through the year to capture the various menu cycles and seasonal changes in eating. 3-day intakes accurately indicate usual intake of macro-nutrients in free living people. In the aged care setting there is little opportunity for consumption of other foods by residents as foods prepared outside the facility are not allowed to be consumed on site and there is minimal opportunity to purchase foods from outside the facility. Audits of study food deliveries or food orders will be performed to determine the amount of dairy food delivered to each facility. All analysis will be performed using Foodworks dietary analysis program (XYRIS Software, QLD).

Recruitment Several aged care providers have expressed interest in participating, and based on the "DPS Guide to Aged Care" over 200 facilities with 45 + bed capacity are in the Melbourne metropolitan region. Aged care organisations and management will be approached to participate. If agreeable all residents are provided with participant information sheets and consent forms and residents and their guardians notified of the project via their newsletters. The project is presented to residents at each facility at forums such as at their regular resident's meeting. Participants agreeing to undergo full assessments must be able to understand the requirements of the project and be capable of self-consenting. Consent by a guardian or next of kin are possible for dietary assessments and medical record reviews, as data collection does not require involvement by the participant. We successfully recruited 20 out of 21 facilities using this method in our previous trial. Consent to provide foods to residents and access to fracture and falls data (coded resident number, age and sex) is obtained from facility management. These levels of consent were permissible through the ethics committee of Austin Health in our previous trial. It is anticipated that the study group will consist of females and males in a ratio of 3:1, as this ratio was observed in our previous trial (11). Recruitment will be on-going in that those who fail to complete the trial (e.g. death or move to high care) will be replaced by new residents so that the number of required residents will be maintained.

Fractures, falls and CV events Incident reporting (all falls and accidents including fractures) is compulsory in aged care facilities. All non-vertebral fractures will be verified using hospital records. Lumbar spine fractures will be determined from the lateral lumbar spine DXA scans and are defined as a loss of height of > 20% of a vertebral body relative to the adjacent vertebral bodies. Information about fractures and falls will be collected monthly and resident lists updated accordingly. Fracture and falls details will be collected on all residents. Data collection is on-going for the duration of the trial with data derived from monthly reviews by the research nurse of all documentation relating to incidents. All incident reports will be reviewed and CV events will be noted and verified from hospital medical records.

Serum A qualified pathologist will take fasting morning samples on-site at facilities therefore one facility can be completed per day. Screening bloods will be analysed to determine inclusion/exclusion. Remaining blood samples will be frozen and batched analysed (i.e. samples from each time point per person analysed together). Fasting blood samples will be analysed for serum 25(OH)D; chemiluminescent immunoassay (CLIA) (Liason, DiaSorin, Stillwater, USA), calcium; indirect potentiometry (SYNCHRON LX, Beckman Coulter Inc. USA), parathyroid hormone (PTH); CLIA (DPC Immulite 2000, Los Angeles, USA), albumin, N-Mid Osteocalcin (OC), total procollagen type 1 amino-terminal propeptide (PINP) and c-terminal telopeptide (CTx); electrochemiluminescence immunoassay (Elecsys 1010 Analytics, Roche Diagnostics, Germany, CV). The intra- and inter-assay CV's for serum measures were 7-13% (50). Serum will be used to determine fasting lipids and ApoA & B, and other CV and health markers. These samples will be frozen and batch analysed. Serum samples are collected at 0, 3 and 12 months.

Bone structure and body composition Bone structure and cortical porosity will be assessed at the distal tibia and radius using high resolution micro-pQCT (Xtreme CT;Scanco Medical AG, Bassersdorf, Switzerland, CV < 0.6 – 7.4%). Parameters of interest are: trabecular density, thickness, number and separation; cortical thickness and density. Cortical porosity is determined using a fully automated image processing station (Strax1.0). This new technology developed in-house allows for the first time, an accurate and reproducible quantification of intra-cortical pores as small as 50 microns —below the nominal resolution of the HR-pQCT (82 microns). Quantification of porosity at such a low scale will allow a more comprehensive assessment of the effects of enhanced dairy food intake on bone micro-structure and strength. Bone density (BMD) will be assessed at the lumbar spine, femoral neck and total body using DXA (Prodigy, Version 7.51, GE Lunar, Madison, WI, CV = 1%). Lateral lumbar spine scans

will be done to determine the presence or absence of vertebral fractures defined as a reduction in vertebral height of > 20% relative to the adjacent vertebral bodies. Total body scans will be used to assess changes to body composition and to estimate lean muscle mass and its distribution (i.e. central and appendicular lean and fat mass). Sacropenia will be determined from this data. Residents will be transported in groups of up to 4 to the Heidelberg Repatriation Hospital using an oversized taxi and under the supervision of a qualified nurse. Morning and afternoon sessions will be available three days per week so up to 24 participants can be tested weekly. Scan times are approximately 3 minutes each. Bone measures will be performed at baseline and 12 months, with the option of additional bone measures at 24 months pending budget and progress.

Follow up of residents

Attrition by death or movement to high care is monitored by monthly updates of resident lists and documented. Cause of death will be determined from hospital medical records.

Cardio-vascular and sarcopenic end points

Mortality rates in this age group will be approximately 10% per year. Of these about 5% per year are likely to be cardiovascular deaths with an additional 5% annual rate of non-fatal CV events. The primary "cardiovascular" end-point would be any of the following (i) all-cause mortality (ii) acute myocardial infarction (proven with doubling of serum troponin) or (iii) stroke. The chosen composite end-point would require neither an Event Adjudication Committee nor brain imaging, thus making this additional component of the study easy to include, and also more generalisable to the general outcome of this elderly population. The primary event rate would be approximately 20% over 2 years. Equivalence statistics requires wider 97.5% confidence interval than the usual superiority methods that require 95% CIs, but in only one direction (51). A clinically significant difference between groups in the primary end-point could be as small as 5%. A stringent alpha of 1% would be very tight proof of equivalence, even though a less emphatic 2% is easier to reach and commonly used.

Blood pressure will be determined using a random zero sphygmomanometer with an average of 3 readings over 5 minutes in the seated position. Blood flow is measured using a tonometry that involves the placement of pressure sensors on the skin over arteries.

Physical function

Balance function is assessed by measuring dynamic stability using a stadiometer (Good Balance Platform System™, Metitur Ltd. Finland). Participants standing on an equilateral triangular force platform, the stadiometer converts shifts in weight to digital data by which a quantitative assessment of maintenance of balance is obtained. Balance will be assessed in different standing positions. This will be performed during the visit to the Heidelberg Repatriation Hospital for bone and body compositional assessments. Knee flexion, extension, hip abduction, adduction and ankle plantar and dorsi flexion will be assessed using a Nicholas Manual Muscle Tester. Isometric hand-grip strength using a hand-grip dynometre will be used to assess muscle strength as it correlates with lower leg strength (52). Gait speed over 6 metres will be used as an indicator of function and is predictive of disability (53). Risk of malnutrition will assessed using the Mini Nutrition Assessment tool. The same researcher will perform all measures, before and after the intervention period. These measures will be performed at the facilities.

Power calculations

This study is restricted to low-level aged care residents as falls and fracture rates are highest in this group. Based on a fracture rate of 7% per year in Australian elderly living in low level aged care, (14% over the two year study period) 2000 elderly residents will be needed to detect a 30% reduction in fractures with 80% power (4, 9). Sanders and Nicholson report a two-year fracture incidence of 13.4% in elderly female aged care residents (54). The minimum number of beds per

facility to be recruited is 50 (maximum 120). Based on an estimation of an average of 60 residents per facility, 60 facilities provide a minimum of 3600 residents, therefore with a 20% yearly attrition rate, 2400 residents will remain by the completion of the 2 year study, providing the sample size needed to detect a fracture rate reduction of 30% at 80% power, $p < 0.05$.

Randomising individuals is not feasible in this study. However, Rapp et al. reported that clustering accounted for only 0.4% of the variance in outcomes related to falls (55). The sample size for a cluster clinical trial is dependent on the intracluster correlation coefficient (K). Under the hypothesis that the intervention reduces the risk of fracture by 30% and the above statistical assumptions, the required sample size as a function of K can be seen in the following table (56).

Table 1. Number of clusters according to sample size per group (N) and the intracluster correlation coefficient (K) - based on 2-year rate of 14% + 30% reduction

N per group	K=0.50	K=0.25	K=0.20	K=0.15	K=0.10	K=0.05
50	52	28	25	22	21	20
100	43	18	15	13	12	11
200	38	14	11	9	7	6

An intracluster correlation coefficient (K) of 0.20 has been calculated from falls data acquired during the year prior to intervention of our previous trial in low-level aged care residents. We are recruiting 60 facilities with a minimum of 50 residents per facility to account for attrition. Attrition in the prior trial was 40% over 2 years (20% per year). To account for this attrition we will replace lost-to-follow-up individuals with new individuals to ensure the sufficiency of sample size in each cluster. Therefore, recruitment within a facility will be on-going for the duration of the project. Time to first fracture in supplemented and un-supplemented residents will be recorded.

Facilities are matched by location so residents are derived from similar areas, and then randomly allocated to intervention or control. Therefore differences between facilities are not anticipated as all residents entering aged care facilities undergo assessment prior to admission and facilities do not vary in relation to demographics and health and medical status of residents. Residents with greater needs are placed in high-level care facilities, which are not included in this project. We recruited 20 facilities in close proximity to Austin Health in 3 months in our previous trial.

Fracture risk reduction A 30% reduction in fractures is similar to that observed by Chapuy et al (6). Given that all women and 84% of men consume below the recommended intake for calcium, 75% consume below recommended protein levels and all fractures (vertebral and peripheral) will be documented this is a reasonable efficacy estimation. No comparative trials have been conducted investigating the anti-fracture efficacy of protein or dairy foods in the elderly. We anticipate that this intervention will be as effective as the study by Chapuy et al as these residents are deficient in both nutrients and many will have prevalent fractures. The required sample size can be achieved by recruiting 60 aged care facilities (half of which will be supplemented).

Bone strength, bone turnover and secondary endpoints Sample sizes for secondary outcome measures are calculated from our previous study in low-level aged care residents. They are as follows:

N = 70 per group to detect an 8% difference in cortical thickness (HR-pQCT)
N = 75 per group to detect a 20% difference in bone turnover (serum marker osteocalcin)
N = 35 per group to detect a 5% difference in proximal femoral BMD (DXA)
N = 75 per group to detect a 20% difference in PTH

We will recruit 240 participants (120 per group) to undergo full assessments (blood, bone structure) to account for attrition.

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Final study protocol

Study design This two-year cluster-randomised controlled intervention involved recruitment of 60 residential aged-care facilities housing 7195 older adults in metropolitan Melbourne and regional Victoria, Australia, between December 2013 to August 2016. To ensure similar standards of care, we recruited only facilities accredited with the Australian Aged-Care Accreditation Agency that housed predominantly ambulant residents. These facilities are similar to residential care in the UK and assisted care facilities in the USA. Facilities recruited were representative of charitable, private and religious organisations with an even distribution of small (up to 50 beds), medium (51-100 beds) and large (>100 beds) facilities (supplementary figure S3). The ratio of women to men, and age of residents is representative of the national average.(10)

Inclusion criteria Randomisation was by facility, not by individuals. For inclusion, facilities were required to provide no more than 2 dairy servings daily, which was assessed from menu audits as this level of provision is associated with dietary intakes of < 1 g per kg body weight and 600 mg calcium daily.(11) Vitamin D adequacy is maintained in residents through routine supplementation as foods are not vitamin D fortified. Only permanent residents were included in data analyses i.e., data from respite residents were excluded.

Randomisation procedure The unit of randomisation was facility as the intervention was delivered to all residents by the food service at each facility. Eligible facilities were randomly assigned in a 1:1 ratio to either intervention (n = 30) or control (n = 30), with the latter maintaining their existing menus. The randomisation was conducted with the use of a computer with block sizes being varied according to organisation (to ensure similar procedures and policies) and was stratified by geographical location (to ensure similar socio-economic status). This was done by a statistician independent of the study who provided the concealed group allocation to the principal investigator, (SI), who in turn conveyed this allocation to the facility. SI was not involved in any data collection. An organisation may have 2 to 10 facilities, and randomisation was done within an organisation.

Consent Facility managers consented to provide de-identified details of age and sex of residents, and access to all incident reports including those for falls and fractures. Reporting of all incidents of any nature is a mandatory requirement of all accredited aged-care providers. Incident reports are regularly audited by the Accreditation Agency. If breaches are observed, facilities are sanctioned with potential for accreditation to be revoked and government funding terminated. Falls (time, location, circumstances and outcome / injury) and fractures and other adverse events were verified from these incident reports. These reports were maintained at all facilities. An independent medically trained person blinded to study allocation verified fractures using hospital x-rays and x-ray reports. Residents and families were informed of the study during regular meetings. A subset of 371 residents from all facilities voluntarily consented to have dietary intake recorded, medical records reviewed, blood sampling, and measurement of body composition, bone mineral density and bone microarchitecture performed. An additional 345 residents were consented by a next of kin to allow dietary intake to be recorded and medical records reviewed.

Intervention Dairy foods were classified using the Australian Dietary Guidelines with a 'serving' defined as milk (250 ml), yoghurt (200 grams) and cheese (40 grams).(12) Lactose free options were provided to accommodate the few participants (<0.001%) with reported lactose intolerance. Butter, cream and ice-cream were not provided as they contain little calcium or protein. All facilities prepared and cooked foods on-site. Intervention facilities were assigned a food service dietician to assist food service staff to increase the provision of dairy foods at all meals and snacks. Methods used to increase dairy foods included milk powder to fortify milk used in recipes and beverages. Dairy-based desserts and snacks were offered in place of less nutritious foods such as

cakes and biscuits. Foods provided were based on the preferences expressed by the residents at intervention sites.

Dairy foods were provided in-kind by Fonterra International (New Zealand) and distributed by a commercial food distribution company not associated with the project (Bidfoods, Australia). A single distributor ensured accurate recording of costs for all dairy foods provided, with invoices used to verify compliance data. During dietary assessments, foods and beverages were weighed on a food scale (±1g) (Sohnele Page Profi, Germany) at all facilities. During 2-days every three months, dieticians assessed compliance using the validated visual estimation of plate-waste with data collected from 55,000 foods and beverages during the study.(13) Nutrient intakes were calculated using a nutritional analysis software (FoodWorks, Australia), or the Australian food composition database NUTTAB 2010.

Data monitoring Data safety monitoring was carried out by the Study Trial Review Board who were provided with quarterly reports.

Outcomes As per the approved study protocol, all pre-specified primary and secondary outcomes have been reported. The primary outcome was time to fragility fracture. Secondary outcomes were time to fall and changes in bone morphology and biochemistry. The tertiary outcomes of all-cause mortality and changes to body composition are also reported. Fasting morning serum samples were obtained from 189 residents at baseline and 12 months for measurement of 25-hydroxy-vitamin D (baseline only), C-terminal telopeptide of type 1 collagen (CTX; measure of bone resorption), procollagen type 1 N propeptide (P1NP; measure of bone formation) and parathyroid hormone (Roche Cobas E170) and insulin-like growth factor 1 (LIASON) (supplementary figure S1).

Body composition and bone morphology were assessed at baseline and 12 months in 72 residents (supplementary figure S2). Total and appendicular (arms and legs) lean mass and fat mass were determined from total body scans and bone mineral density (BMD) was measured at the lumbar spine and femoral neck using dual x-ray absorptiometry (Prodigy, GE Lunar, Madison, WI, CV=1%). Volumetric bone mineral density (vBMD; the amount of bone contained within the external volume of bone, g/cm^3) was measured at the distal tibia and distal radius using high-resolution peripheral quantitative computed tomography (Scanco Medical AG, Switzerland, CV 0.5–4.0%).(14) Cortical porosity was determined using automated image processing (StrAx1.0, Straxcorp, Melbourne, Australia).

Blinding and sample size Once a facility was randomised, only the principal investigator, food service research dieticians, facility managers and food service staff were aware of the allocation. Data acquisition and analyses were carried out by staff blinded to group allocation (SP, XW, MB, AGZ, TN). Residents were blinded to the study; permission to conduct the study was obtained from the aged-care provider and facility managers. Some of the intervention strategies were not visible e.g., fortification of milk with milk powder, or modification of recipes. Some residents may be aware of some changes such as provision of cheese and biscuits for snacks, but not the reason for the changes.

The sample size was determined based on a hypothesized effect size and intra-cluster correlation coefficient (*r*). Under the hypothesis that the intervention reduces the risk of fracture by 30%, based on prior anti-fracture calcium / vitamin D intervention in this setting, and that *r* ranges from 0.10 to 0.50, the sample size needed was 25 to 50 residents per facility and 25 facilities per arm to achieve the power of 80%.(6) From falls data, an *r* of 0.20 was used to calculate the sample size.(15) To account for ~20% annual attrition, we recruited 60 facilities with a minimum of 50 residents per facility.

At study commencement 3980 permanent residents were living in the participating facilities. We refer to these residents as the inception cohort. To ensure the required sample size was maintained, recruitment continued throughout the 24-months so data from residents admitted to facilities that replaced initial residents lost to follow up due to death or discharge were included in analyses. We refer to these residents as the replacement cohort. In total an additional 3215 residents were admitted to facilities after the study had commenced. Details of new residents and those lost to follow-up were obtained from admission and discharge records from each facility.

Analyses Baseline data were expressed as mean (± standard deviation, SD) with the unit of analysis being clusters. Fracture incidence, falls and death were expressed per 100 person-years follow-up. The product limit (Kaplan–Meier) method was used to determine the cumulative risk of an event. There were no missing data for these primary and secondary outcomes. The duration of follow-up was based on date of study entry to date of an event. When no event occurred, follow-up duration was date of study entry to date of study termination.

As individuals were 'nested' within clusters (facilities), the primary analysis was based on the mixed-effects Cox's proportional hazards model; effects of intervention, age and sex were fixed effects, and the facility considered the random effect. (See Supplement for additional statistical analysis). The results were expressed as a hazard ratio with 95% confidence limits. Model parameters were estimated by the 'coxme' package. Mortality competing risk analysis was also conducted using the Fine - Gray sub-distribution method with the 'cmprsk' package.

Between-group differences in serum biomarkers and measurements of body composition and bone morphology at baseline were tested by the weighted t-test with cluster being the unit of analysis. Biomarkers were log transformed if they were not normally distributed. Effects of intervention were analysed by the mixed-effects model in which the within-person change in outcome was modelled as a function of treatment or control group, time of follow-up, age, weight and sex. All analyses used the R Statistical Environment.

Amendments to protocol and statistical analysis plan Initially, facilities were matched only by location to account for socioeconomic status. We also accounted for organisations as they contributed varying numbers of facilities and had different policies and procedures. Two instead of three-day diets were quantified as this was adequate to capture regular intakes.(11) Osteocalcin was not assessed as sufficient information is obtained from CTX and P1NP. Only all-cause mortality was used as a tertiary outcome as cardiovascular events were not obtainable and causes of death were poorly documented. Bayesian analyses and imputations were not included as there were no missing values for falls and fracture outcomes.

Ethics The study was approved by the Austin Hospital Human Research Ethics committee (Project Number 04958) and is recorded on the Australian New Zealand Clinical Trials Registry (ACTRN12613000228785, www.anzctr.org.au).

Patient Public Involvement Aged-care residents, providers and food service staff were consulted following the initial feasibility study that guided the design of this intervention (16). The manuscript was read by non-academics.

Dissemination Once published, a national (and international) promotional strategy will be implemented using mainstream and social media. Training for food service staff to implement the methods used in the trial is planned. Outcomes from the trial will be used to improve policy and good clinical practice. Participants and participating facilities will be provided with plain language statements and copies of the publication.

Study Protocol and Amendments

Australia New Zealand Clinical Trial Registry Protocol	Human Research Ethics Committee Approved Protocol	Amendment to Human Research Ethics Committee approved protocol	Deviation	Reason and Time*
Primary Outcome				
Time to fracture	Time to fracture	Time to fracture	None	
Secondary Outcomes				
Time to fall	Time to fall	Time to fall	None	
Bone structure & density measured at baseline, months 12, 24	Baseline, M12	Baseline, Month 12	Month 24 excluded	High attrition rates.
Bone biochemistry measured at baseline, months 3, 12, 24	Baseline, Month 3, Month 12	Baseline, Month 12	Month 24 excluded Month 3 excluded Osteocalcin not measured.	High attrition rates. Baseline - month 12 data aligned with bone and body composition outcomes. Sufficient information obtained from bone resorption (CTX) and formation (P1NP) markers.
Tertiary Outcomes				
Metabolic regulation measured at baseline, months 3, 12, 24	Baseline, M3, M12	Baseline, M12	Month 24 excluded Month 3 excluded Outcome status	High attrition rates. Baseline and month 12 data aligned with bone and body composition outcomes. Registry mistakenly lists all outcomes as secondary.
Body composition measured at baseline, months 12, 24	Baseline, M12	Baseline, M12	Month 24 exclude Outcome status	High attrition rates. Registry mistakenly lists all outcomes as secondary.

Malnutrition measured at baseline, months 3, 12	Baseline, M3, M12	Baseline, M12 Weight loss reported.	Month 3 excluded Outcome status	Baseline and month 12 data aligned with bone and body composition outcomes. Registry mistakenly lists all outcomes as secondary.
All-cause mortality	Included (i) all-cause mortality (ii) acute MI (proven with doubling of serum troponin) or (iii) stroke.	All-cause mortality	All-cause mortality Outcome status	Data on cardiovascular events not obtainable. Cause of death poorly documented. Registry mistakenly lists all outcomes as secondary.
Exploratory Outcomes				
Muscle strength & function measured at baseline, months 3, 12, 24	Baseline, M3, M12	Baseline, M12	Month 24 exclude Outcome status	High attrition rates. Registry mistakenly lists all outcomes as secondary.
Quality of Life, ADL's, Health status, depression measured at baseline, month 3, 12	Baseline, M3, M12	Baseline, M12	Outcome status	Registry mistakenly lists all outcomes as secondary.
Other				
Inclusion criteria	Intake <600mg cal/day, <1g/kg BW Pro	Facilities provide < two dairy servings daily	Facility-based than individual-based	< 2 servings of dairy daily associated with intakes of < 1 g per kg BW and 600 mg Ca/d.
	Males not hypogonadal (testosterone >10 nM)	Testosterone not measured.	Not measured	Not feasible.
	Not vitamin D deficient (serum 25(OH)D levels > 25 nmol/L)	Vitamin D status and medical record review assessed in consented residents	Measured in subset of consented residents	No feasible. Vitamin D supplementation is routine in aged-care.
	Free of disease or			

	medication use known to affect bone			
Randomisation	Facilities matched by location	Facilities matched by location & organisation	Include organisation	To account for different policies and procedures.
Dietary Intake	3 days every 3 months.	2 days every 3 months.	Excluded 3rd day	Two days was adequate to capture regular intakes.
Reporting of CV events	CV events verified from hospital medical records	Cardiovascular events not monitored.	Not recorded	Data not obtainable.

* Decision for each deviation was made prior to study commencement.

Original Statistical Analyses

Statistical analysis

The primary outcome of this study is the time to fragility fracture, and the secondary outcome is falls. The incidence rate of outcomes will be expressed as the percentage of events per years of follow-up, taking into account the censorship of follow-up data. Kaplan – Meier estimates will be used to obtain the proportion of subjects who would have an event during the follow-up period. The Cox's proportional hazards model will be used to examine the effect of intervention on fracture risk according to the intention-to-treat principle. Occurrences of outcomes will be compared with the use of hazard ratio and 95% confidence limits. Since the events of fracture and fall are expected to be correlated (i.e., most fractures result from falls) we will use the multi-state Cox-Markov model to account for the correlation (57). The R statistical environment will be used for the statistical analysis.

We will apply modern statistical methods to deal with attrition in this cluster randomised clinical trial. Two main methods for dealing with missing outcomes are Bayesian analyses and imputation. In the Bayesian approach, we will generate the posterior distribution of outcome using Monte Carlo methods (58). Combining this estimate with the known outcome provides a numerator for estimating the incidence of fractures and falls. Under certain assumptions about the missing data, it has been shown that valid inferences can be obtained through a Bayesian analysis. In the imputation approach, missing data will be imputed with one or more suitable estimates prior to the analysis according to the methods described by Little and Rubin (59).

The distribution of continuous covariates e.g. bone turnover markers will be checked for normality by the standard Shapiro's statistic test. Differences in baseline covariates between treatment groups will be tested by unpaired t-test (for continuous variables) or Binomial test (for categorical variables). The effect of intervention on variables e.g. bone remodelling will be analysed by the mixed-effects model, in which the change in bone markers will be modelled as a function of treatment group, time of follow-up, and covariates. The R statistical package will be used for the mixed-effects analysis. Data were presented as mean ± SD unless stated otherwise. The p-value less than 0.05 is considered statistically significant, but values < 0.1 will be reported to indicate trends.

Final Statistical Analyses

Outcomes As per the approved study protocol, all pre-specified primary and secondary outcomes have been reported. The primary outcome was time to fragility fracture. Secondary outcomes were time to fall and changes in bone morphology and biochemistry. The tertiary outcomes of all-cause mortality and changes to body composition are also reported. Fasting morning serum samples were obtained from 189 residents at baseline and 12 months for measurement of 25-hydroxy-vitamin D (baseline only), C-terminal telopeptide of type 1 collagen (CTX; measure of bone resorption), procollagen type 1 N propeptide (P1NP; measure of bone formation) and parathyroid hormone (Roche Cobas E170) and insulin-like growth factor 1 (LIASON) (supplementary figure S1).

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Statistical analyses protocol amendments

Original protocol:	Two main methods for dealing with missing outcomes are Bayesian analyses and imputation. In the Bayesian approach, we will generate the posterior distribution of outcome using Monte Carlo methods (58).
Amended protocol:	Bayesian analyses and imputation not included as there were no missing values for fracture and falls outcomes.